PROTEINS AND USES

FIELD OF THE INVENTION

[0001] The present invention relates to stabilising transient protein:protein interactions using binding proteins. Pharmaceutical compositions comprising the binding proteins are also provided. The invention also relates to use of the binding proteins, including use in delivering cargo to a target cell and use in cross-linking target cells. Various methods are further provided, including methods of specifically targeting cells using the binding protein, methods of switching on a biological effect and methods of cross-linking two cells.

BACKGROUND OF THE INVENTION

[0002] In cancer, most tumour associated antigens are also expressed on normal peripheral tissues and cells, albeit at disparate density. It is commonly believed that the dual targeting of two antigens on the same cell leads to improved target selectivity over normal tissues that express only one or low levels of both antigens.

[0003] One means of targeting two antigens on a tumour cell is via a bispecific antibody (i.e. with one arm of the antibody recognising one target antigen and the second arm of the antibody recognising a second target antigen). However, as shown in Mazor et al mAbs, 7, 461-469, dual targeting by bispecific antibodies is not always sufficient to promote efficient target selectivity. Bispecific antibodies may still recognise cells expressing only one of the two target antigens. Mazor et al reported that the affinity of the individual arms of the antibody, as well as avidity and valence, play an important role in targeting. For example, a bispecific antibody composed of an anti-CD4 arm and an anti-CD70 arm recognised cells expressing both CD4 and CD70, as well as CD4 alone. Affinity-reduced variants of the CD4 arm were prepared by introducing mutations into the CDRs. These variants exhibited improved selectively; binding to cells expressing CD4 alone was reduced. However, in generating such bispecific antibodies extensive engineering is required. Bispecific antibodies are also inherently specific for their particular target antigens. Different bispecific antibodies are thus needed to target different antigen combinations.

[0004] Another technique for recognising multiple antigens on a target cell is described in WO 2013/104804. Here, a targeting moiety for a first antigen "A" is linked to a non-active fragment of a functional domain. A second targeting moiety for antigen "B" is then linked to a second non-active fragment of the functional domain. When both antigens are present on the cell surface, dimerization of the two fragments results in formation of a functional domain.

[0005] One fragment may be a V_L domain of an antibody and the other fragment may be a V_H domain of an antibody thus resulting in an scFv when both antigens are present on the target cell. There are, however, also downsides to this approach in that V_H and V_L by themselves can have stability issues and the system may be partially leaky. Separate V_H and V_L domains may also be prone to aggregation.

[0006] There exists a need in the art to provide improved means to specifically identify and target cells (e.g. immune cells and tumour cells) with minimal off target effects.

[0007] The present inventors have identified a new system with utility, amongst other things, in specifically targeting multiple antigens on cells of interest. The system harnesses transient protein:protein interactions (PPIs), and in particular uses junctional binding proteins which recognise two components of a transient PPI.

[0008] An example of a transient PPI, which plays a fundamental role in the patho-physiology of several diseases, is the interaction between IL-6 and its specific receptor gp80, (also known as CD126). IL-6 binds to gp80 to form a heterodimer; this first step is characterized by fast association and dissociation phases (Ward et al. The Journal of biological chemistry 271, 20138-20144 (1996)). The IL-6–gp80 complex then can bind to gp130 to form a heterotrimer, which in turn dimerizes to create the active hexameric complex responsible for key downstream signaling events (Boulanger et al Science 300, 2101-2104 (2003)). IL-6 can signal in cis or trans, depending on whether gp80 is cell membrane-expressed or in a soluble form generated by shedding (Mullberg et al. *European journal of immunology* 23, 473-480 (1993)). Both forms of gp80 are active and bind IL-6 (Rose-John et al Journal of leukocyte biology 80, 227-236 (2006)). In vitro data suggest that the trans-signaling pathway is preferentially activated during inflammation (Rose-John et al. *International journal of biological sciences* 8, 1237-1247 (2012)). Unlike gp130, which is expressed in a variety of cells, gp80 is expressed mainly on the membrane of hepatocytes and immune cells (Rose-John et al Journal of leukocyte biology 80, 227-236 (2006)). Neither IL-6 nor gp80 binds to gp130, indicating that IL-6 is presented by gp80 in the appropriate conformation to then recruit gp130 (Rose-John et al Journal of leukocyte biology 80, 227-236 (2006)).

[0009] IL-6 mediates an array of immune responses including lymphocyte trafficking, proliferation and differentiation of T cells, antibody production from B cells, liver regeneration, expansion/differentiation of bone marrow progenitors and the broad regulation of inflammatory response (Galun, E. & Rose-John, S Methods in molecular biology 982, 59-77 (2013) and Mihara et al Clin Sci (Lond) 122, 143-159 (2012)). IL-6 is a key mediator in a variety of diseases, including cancer and autoimmunity (Hunter, C. A. & Jones, S. A. *Nature immunology* 16, 448-457 (2015)). As such, IL-6 has been targeted directly (IL-6 blocking antibodies) or indirectly (gp80 blocking antibodies; gp130-Fc) for therapeutic interventions against autoimmune disorders and cancer (Rose-John et al Expert opinion on therapeutic targets 11, 613-624 (2007), Calabrese, L. H. & Rose-John, S. Nature reviews. Rheumatology 10, 720-727 (2014), Shaw, S. et al. Discovery and characterization of olokizumab: a humanized antibody targeting interleukin-6 and neutralizing gp130-signaling. mAbs 6, 774-782 (2014), Atreya, R. et al, Nature medicine 6, 583-588 (2000), Nishimoto, N. et al. Blood 106, 2627-2632 (2005) and Milagre, C. S. et al. Cancer research 75, 1255-1264 (2015)).

SUMMARY OF THE INVENTION

[0010] The present invention provides:

[0011] A binding protein specific for a junctional epitope created by a transient protein:protein interaction, which binding protein stabilises the protein:protein interaction.

[0012] The present invention also provides an isolated polynucleotide encoding one or both chains of an antibody or fragment thereof of the invention, a vector comprising the polynucleotide, a host cell comprising the vector and a method of producing an antibody or fragment thereof of the